Planta (2006) 224: 339–346
DOI 10.1007/s00425-005-0217-5

ORIGINAL ARTICLE

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Factors modulating the levels of the allelochemical sorgoleone in *Sorghum bicolor*

Received: 28 November 2005 / Accepted: 16 December 2005 / Published online: 10 January 2006

Abstract Sorgoleone is the major component of the hydrophobic root exudate of sorghum [*Sorghum bicolor* (L.) Moench]. The presence of this allelochemical is intrinsically linked to root growth and the development of mature root hairs. However, factors modulating root formation and the biosynthesis of sorgoleone are not well known. Sorgoleone production was independent of early stages of plant development. The optimum temperature for root growth and sorgoleone production was 30°C. Seedling development and sorgoleone levels were greatly reduced at temperatures below 25°C and above 35°C. The level of sorgoleone was also sensitive to light, being reduced by nearly 50% upon exposure to blue light (470 nm) and by 23% with red light (670 nm). Applying mechanical pressure over developing seedlings stimulated root formation but did not affect the biosynthesis of this lipid benzoquinone. Sorgoleone production did not change in seedlings exposed to plant defense elicitors. On the other hand, sorgoleone levels increased in plants treated with a crude extract of velvetleaf (Abutilon theophrasti Medik.) root. This stimulation was not associated with increased osmotic stress, since decreases in water potential (Ψ_w) by increasing solute concentrations with sorbitol reduces sorgoleone production. Sorgoleone production appears to be constitutively expressed in young developing sorghum plants. Other than with temperature, changes in the environmental factors had either no effect or caused a reduction in sorgoleone levels. However, the stimulation observed with velvetleaf root crude extract suggests that sorghum seedlings may respond to the presence of other plants by releasing more of this allelochemical.

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Keywords Allelopathy · Elicitation of natural products · Essential oil · Lipid resorcinol

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal grain crop grown throughout the world (Doggett 1988), but primarily in the semiarid areas of Africa, India, China, and South America (Subudhi and Nguyen 2000). It is also used in the United States as green manure or as cover crop (Einhellig and Rasmussen 1989; Weston 1996). Evidence for the allelopathic effect of sorghum was first observed in crops grown in rotation with sorghum (Breazeale 1924) and subsequently confirmed in several studies (Putnam et al. 1983; Forney et al. 1985; Einhellig and Rasmussen 1989). Later work identified many compounds produced by sorghum roots that putatively played a role in the allelopathic potential of this species. While early studies suggested that several classes of water-soluble compounds, particularly phenolics, were involved (Guenzi et al. 1967; Lehle and Putnam 1983; Alsaadawi et al. 1986; Panasiuk et al. 1986), more recent work identified an oily exudate containing the lipid benzoquinone sorgoleone (2-hydroxy-5-methoxy-3-[(8′*Z*, 11′*Z*)-8′, 11′, 14′pentadecatriene]-*p*-benzoquinone) as the leading source of the allelopathic properties of sorghum (Netzly et al. 1986).

The herbicidal activity of sorgoleone is strongest on small-seeded weeds (Einhellig and Souza 1992; Rimando et al. 1998; Netzly and Butler 1986; Nimbal et al. 1996a; de Souza et al. 1999; de Almeida Barbosa et al. 2001). Large seeded weeds tend to be less sensitive to sorgoleone. These plants may avoid the herbicidal effect by having lower absorption and translocation or faster metabolic degradation of the allelochemical, or simply by having roots rapidly growing beyond the zone of the sorghum rhizophere where sorgoleone accumulates. This lipophilic quinone is active on several molecular target sites, inhibiting photosynthesis by competing for the plastoquinone binding site on PSII (Rimando et al.

1998; Gonzalez et al. 1997; Nimbal et al. 1996b; Einhellig et al. 1993), affecting mitochondrial functions (Rasmussen et al. 1992), inhibiting the enzyme *p*-hydroxyphenylpyruvate dioxygenase (HPPD) (Meazza et al. 2002), and interfering with root H^+-ATPase and water uptake (Hejl and Koster 2004). Whether the allelopathic potential associated with sorgoleone is the result of inhibiting one or more of these molecular target sites is still unknown.

Sorgoleone and its 1,4-hydroquinone form account for 90% of the oily root exudate of sorghum root hairs. The remaining 10% contains several minor congeners varying in the substitutions in the aromatic ring, and/or in the number of carbons and the level of unsaturation in the tail (Fate and Lynn 1996; Rimando et al. 1998; Kagan et al. 2003). All these variants of sorgoleone appear to contribute to the overall allelopathic potential of sorghum (Kagan et al. 2003; Rimando et al. 2003).

The biosynthetic pathway of sorgoleone has been characterized (Fate and Lynn 1996; Dayan et al. 2003). It involves the convergence of two metabolic pathways. A fatty acid synthase and fatty acid desaturases produce the obligatory 16:3-CoA that subsequently serves as the starter unit of a specialized polyketide synthase. The resulting lipid resorcinol is acted upon by a SAM-dependent O-methyltransferase and a P450 monooxygenase to produce the reduced (hydroquinone) form of sorgoleone (Dayan et al. 2003).

It is also postulated that the biosynthesis of sorgoleone is intrinsically linked to the presence of living root hairs (Czarnota et al. 2001; Yang et al. 2004). Moreover, it has been demonstrated that root hair formation was inhibited by excess water (Hess et al. 1992; Yang et al. 2004) and that ethylene promoted root hair development under such conditions. But, unlike in Arabidopsis (Dolan 2001), ethylene did not appear to be the only positive regulator of root hair initiation and elongation in sorghum.

In spite of the increasing knowledge of the cellular localization and the biosynthetic steps involved in the formation of sorgoleone, relatively little is known with regard to the factors modulating its biosynthesis. In order to better understand the factors affecting levels of sorgoleone exuding from root hairs, the effects of different environmental conditions on sorgoleone production in developing young sorghum seedlings were examined. Factors tested included plant age, temperature, blue, red, and far-red lights, pH, osmotic stress, mechanical pressure (g cm^{-2}), and natural and synthetic elicitors of systemic acquired resistance (SAR).

Materials and methods

Plant material and growth conditions

Seeds of the sorghum cultivar SX17 (*S. bicolor* X *S. sudanense*) were purchased from DEKALB Genetics (DeKalb, IL), surface-sterilized by soaking for 20 min in 10% bleach (0.615% sodium hypochlorite), and rinsed with copious amount of deionized water. For most assays, 20 seeds (ca. 500 mg) were placed in 20×100 mm sterile Petri dishes over the surface of sterile Whatman #1 filter paper (90 mm diameter). Three milliliters of sterile water was added to the dish, and the seeds were covered with a second sterile filter paper. The top lid was placed upside down to provide some mechanical pressure over the top of the developing seedlings. The dishes were sealed and incubated in the dark at 30°C. Sorgoleone was extracted from 7-day-old seedlings in all experiments unless otherwise indicated. Each experiment had three to five replications and was repeated over time. All procedures were executed under low-intensity green light to prevent the formation of anthocyanins by sorghum roots and photodegradation of the samples during extraction.

Extraction and quantification of sorgoleone

Roots were excised from the 7-day-old sorghum seedlings and immersed in $CHCl_3$ for 3 min. The roots and root debris were removed and the organic solvent was collected and dried in vacuo (Büchi ROTAVAPOR R-124, Brinkmann Instruments Inc., Westbury, NY) at 40°C. The weight of the total crude extract was recorded prior to compositional analysis by HPLC.

Analysis of Sorgoleone by HPLC

Sorgoleone in the root exudate was quantified by HPLC as described before (Czarnota et al. 2003a). The HPLC system was composed of Waters Associates (Milford, MA 01757, USA) components, which included a Model 510 pump, a Model 712 autosampler, a Millenium 2010 controller, and Models 470 fluorescence and 990 photodiode spectrophotometric detectors. The column was a 3.9×300 mm (ID) μbondapak C18 reversed phase (Waters Associates). The isocratic solvent system consisted of 80% acetonitrile with 0.1% TFA. The injection volume for each sample was 25 μl and each injection lasted for 10 min. The amount of sorgoleone in each extract was quantified at A_{287} based on a calibration curve obtained with pure sorgoleone.

Light treatments

Sorghum seeds were germinated in a tri-chromatic E-30LED plant growth chamber (Percival Scientific Inc. Perry, Iowa 50220 USA). The wavelengths generated by the LED systems were 470 nm for blue light, 670 nm for red light, and 735 nm for far-red light. The light intensity for the tri-chromatic lamp bank was approximately 470 μmol m^{-2} s^{-1} in total. The seedlings were exposed for 10 min to each respective wavelength (at 50%

maximum intensity) every hour for 7 days. The temperature was maintained at 30°C throughout this experiment.

Temperature treatment

Sorghum seeds were germinated in darkness in a CU-32L plant growth chamber (Percival Scientific Inc. Perry, Iowa 50220 USA) for 7 days at 15, 20, 25, 30, 35, and 40°C.

Effect of mechanical pressure over the seedlings

The glass Petri dishes were set up as described above, except that 50, 100, and 150 g of sand were added inside the top lid, prior to placing them upside down over the seedlings to provide 1, 1.8, and 2.7 g cm^{-2} pressure over the developing seedlings, respectively. The control treatment consisted of seedlings in the glass Petri dishes with the lid right side up (i.e., the surface of the lid coming in contact with the seedlings) resulting in 0.17 g cm^{-2} over the plants. The dishes were sealed in zip-lock bags to prevent evaporation of the water.

Effect of pH

Seedlings were grown in buffers to determine the effect of pH on sorgoleone production. Buffers at pH 4.0 to 6.0 were obtained by mixing various volumes of 200 mM sodium phosphate dibasic and 100 mM citric acid according to Ruzin (1999). The final solutions were diluted to contain approximately 25 mM sodium phosphate. The buffers ranging from pH 6.0 to 8.0 were made by mixing different volumes of 1 M potassium phosphate dibasic and 1 M potassium phosphate monobasic according to Sambrook and Russell (2001) and diluting these solutions to 25 mM potassium phosphate.

Effect of osmotic stress

Seedlings were grown in Petri dishes as described above but in the presence of 0, 100, 250, 500, and 750 mM sorbitol or 10 and 100 mM NaCl. The water potential (Ψ_w) of the sorbitol solutions were −0.25, −0.62, −1.24, and −1.86 MPa at 25°C, respectively. The water potential (Ψ_w) of the NaCl solutions were −0.05 and −0.5 MPa at 25°C, respectively.

Effect of elicitors on root growth and sorgoleone production

Velvetleaf (*Abutilon theophrasti* Medik.) seedlings were grown in growth chambers for 3 weeks. The roots were collected and washed lightly to remove soil. The excised roots were placed in water overnight to extract water-soluble components of the velvetleaf roots. This solution was lyophilized and stored. Chitin (a linear polysaccharide component of pathogenic fungi cell walls) was purchased from Sigma-Aldrich (Milwaukee, WI). Messenger (harpin protein isolated from *Erwinia amylovora*) and ProAct (a complex mixture of harpin N, harpin W, popA, and harpin Z derived from *E. amylovora*, *Ralstonia solanacearum,* and *Pseudomonas syringae*) were purchased from Eden Bioscience Corporation (Bothell, Washington). Actigard (benzothiadiazole) was purchased from Syngenta (Wilmington, DE).

Chitin, Actigard, Messenger, ProAct, and the water-soluble extract of velvetleaf roots were tested in an attempt to stimulate sorgoleone production. Concentrations of chitin, Actigard, Messenger, ProAct, and velvetleaf extract were 10, 0.5, 0.75, 0.75, and 0.5 mg/ml, respectively.

Statistical analysis

All data were analyzed with the SAS Software release 8.0 (SAS Institute Inc., Cary, NC, USA). Analysis of variance was performed for each compound concentration and means were tested with Tukey's test. Standard deviations are also provided to show the variations associated with particular means.

Results

Observation of sorghum roots indicates that sorgoleone is exuded as an oily droplet at the tip of the root hairs (Fig. 1a). While the unspecialized epidermal cells of roots do not appear to produce sorgoleone, epidermal cells that specialize and produce root hairs have the ability to produce sorgoleone. Furthermore, these root hairs do not begin exuding sorgoleone until these specialized cells have stopped elongating (Fig. 1b). Any root possessing root hairs, including primary and secondary roots (Fig. 1c, d), exudes large amounts of sorgoleone.

As expected under normal growth conditions, root biomass (and the total amount of sorgoleone) increases as the plant ages (Fig. 2). However, the production of sorgoleone appears to be constitutive and the levels of sorgoleone remain constant when adjusted per root dry weight.

In this study, the amount of sorgoleone exuded from sorghum root hairs was dramatically affected by temperature. Not surprisingly, low temperatures reduced root growth, but also resulted in lower sorgoleone synthesis (Fig. 3). Optimum sorgoleone production was obtained at temperatures ranging from 25 to 35°C, with maximum levels obtained at 30°C. Root biomass was reduced by 50% when the temperature reached 40°C. Interestingly, sorgoleone levels were even more affected by higher temperature, being only 5.4% of the amount produced at 35°C.

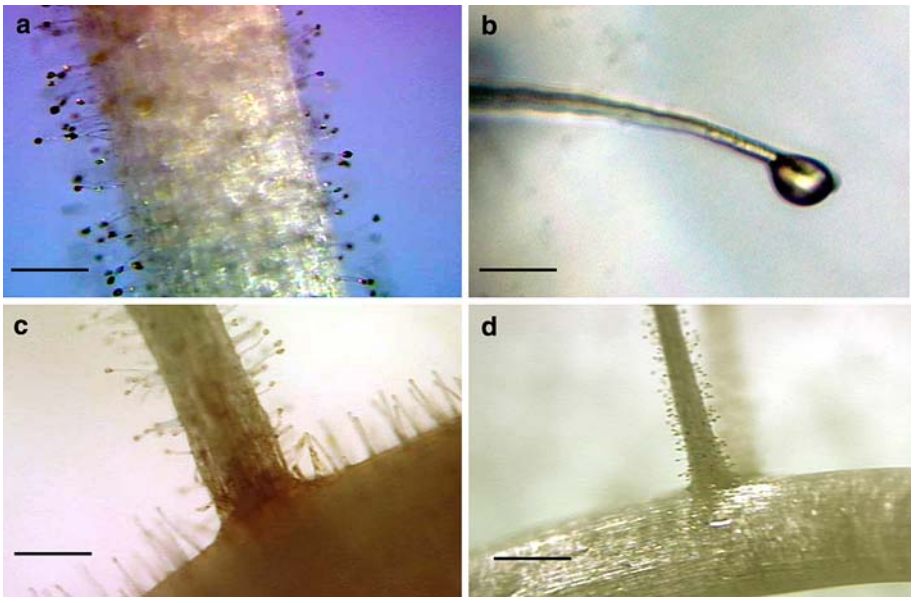

Fig. 1 Photomicrograph of *Sorghum bicolor* roots showing **a** sorgoleone-rich oily exudate secreted from the root hairs (Bar = 80 μm) and **b** closer view of a root hair with sorgoleone exuding at the tip (Bar = 15 μm). Sorgoleone-rich oil from tip of root hairs also exudes from secondary roots originating either from **c** roots (Bar = 125 μm) or **d** stem (adventitious roots) (Bar = 350 μm)

Since growing sorghum seedlings in the presence of light typically represses root growth, the effect of various light wavelengths was tested to determine which component of light might regulate sorghum root growth. Interestingly, none of the light treatments affected root formation (Table 1). However, sorgoleone production was repressed nearly 50% by blue light (470 nm) and 23% by red light (670 nm), relative to dark grown control seedlings. Far-red light (735 nm) had a very small effect.

In order to increase the surface contact between the developing seedlings and the filter papers in the Petri dishes, the top lid was placed 'upside down' so as to apply some mechanical pressure. This resulted in an increase in root biomass. Therefore, different amounts of pressure were applied over the developing seedlings (Fig. 4). While increasing the pressure over the seedling significantly raised root biomass accumulation, the constitutive nature of sorgoleone exudation from root hairs resulted in no net increase in sorgoleone production per root dry weight.

Sorgoleone production increased slightly, though significantly, as the pH decreased (Fig. 5). However, the levels of sorgoleone were greatly reduced, relative to the

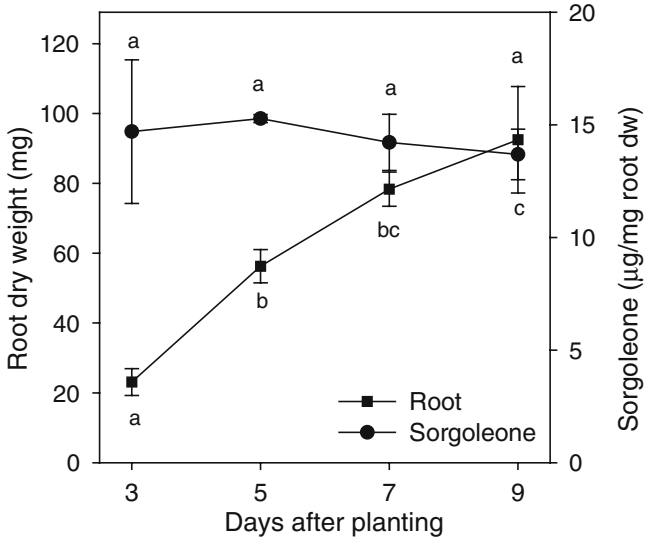

Fig. 2 Effect of plant age on root formation and the production of sorgoleone in *Sorghum bicolor* cv. SX17. Sorgoleone was extracted from seedlings grown in the dark at 30°C. Means with the same letter above (sorgoleone) or below (root) the symbols are not different at $P < 0.05$ according to Tukey's test

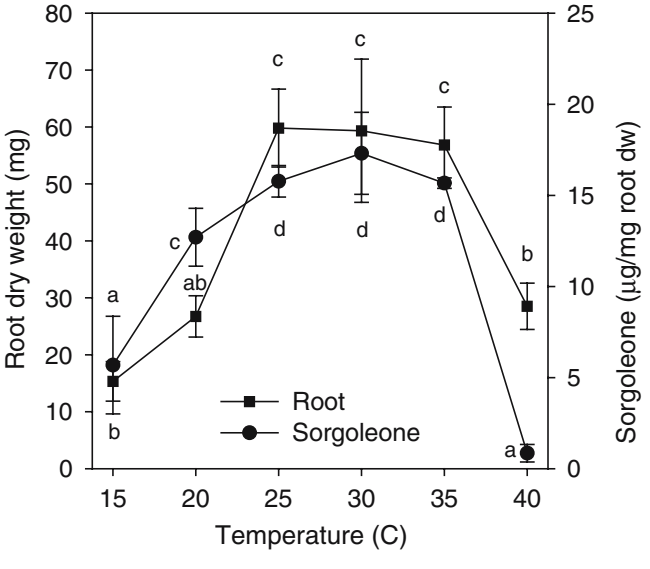

Fig. 3 Effect of temperature on root formation and the production of sorgoleone in *Sorghum bicolor* cv. SX17. Sorgoleone was extracted after 7 days of growth in the dark. Means with the same letter above (root) or below (sorgoleone) the symbols are not different at $P < 0.05$ according to Tukey's test

Table 1 Effect of darkness, blue, red, and far-red lights on root formation and the production of sorgoleone in *Sorghum bicolor* cv. SX17

Light treatment[a]	Root		Sorgoleone	
	(mg dw ± sd)[b]	% darkness	(μg/mg root dw ± sd)[b]	% darkness
Darkness	45.4 ± 1.7 a	–	11.7 ± 0.5 a	–
Blue	50.6 ± 2.0 a	111.4	6.1 ± 0.3 d	52.1
Red	52.7 ± 8.4 a	116.1	9.0 ± 0.3 c	76.9
Far-red	45.2 ± 4.8 a	99.6	10.9 ± 0.6 b	93.2

[a]Seedlings were grown on a 10 min light/50 min dark cycle (except for the control that was maintained in complete darkness). The wavelengths for the blue, red and far-red lights were 470, 670, and 735 nm, respectively
[b]Numbers in columns followed by the same letter are not different at $P < 0.05$ according to Tukey's test

amount extracted from seedlings grown in water without buffer.

Elicitors of plant defense mechanisms did not greatly affect sorgoleone output (Table 2). Treating plants with Actigard repressed sorgoleone production by 30%, whereas treating with chitin and messenger had no effect (Table 2). Exposing sorghum seedlings to ProAct or a velvetleaf extract caused modest elevations in sorgoleone levels. However, these increases were not statistically significant. Nevertheless, in a more elaborate experiment testing various concentrations of a water-soluble extract of velvetleaf, sorgoleone production was significantly stimulated in a dose-dependent manner while reducing overall root biomass (Fig. 6).

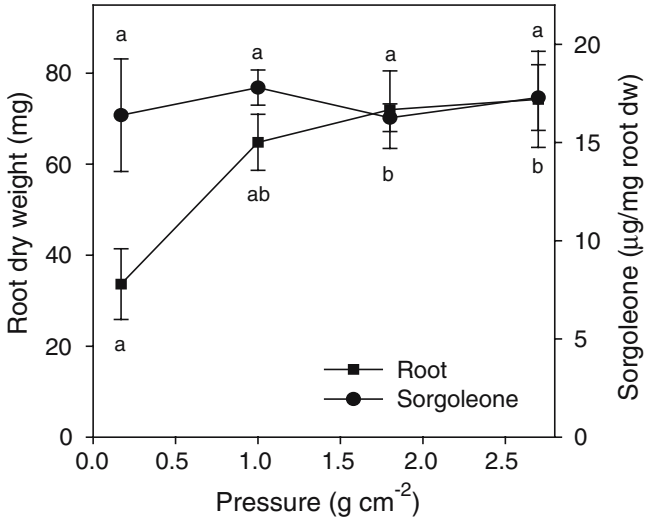

Fig. 4 Effect of mechanical pressure over the germinating seeds on root formation and the production of sorgoleone in *Sorghum bicolor* cv. SX17. Sorgoleone was extracted after 7 days of growth in the dark at 30°C. Means with the same letter above (sorgoleone) or below (root) the symbols are not different at $P < 0.05$ according to Tukey's test

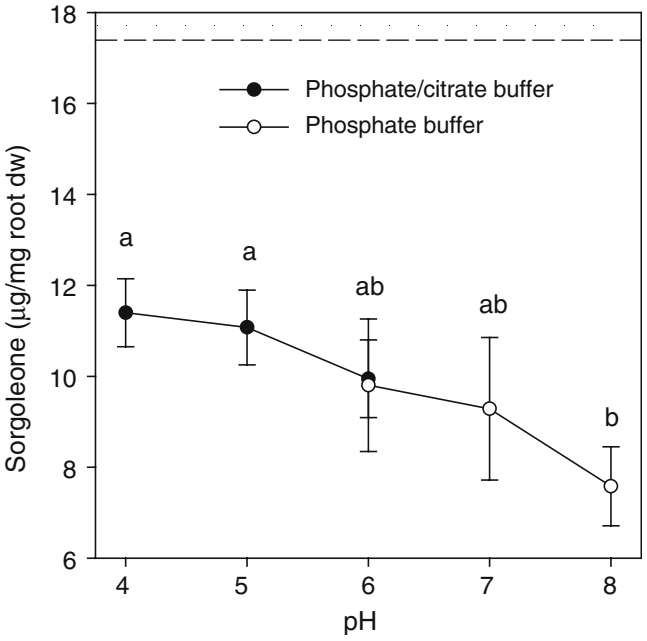

Fig. 5 Effect of pH on the production of sorgoleone in *Sorghum bicolor* cv. SX17. A phosphate-citrate buffer was used for pH ranging from four to six and a phosphate buffer was used for pH six to eight. The *dotted line* indicates the amount of sorgoleone produced by seedlings grown with water only. Means with the same letter above the symbols are not different at $P < 0.05$ according to Tukey's test

The production of sorgoleone and root weight were negatively affected by osmotic stress generated by lowering the water potential of the medium with sorbitol (Fig. 7). The relationship between root weights (or sorgoleone amount) and the water potential of the solution applied to the seedlings was nearly linear. Sorgoleone levels were 10% lower in roots exposed to 0.1 M NaCl and the seed germination was dramatically reduced at 1 M NaCl.

Table 2 Effect of elicitors on root formation and the production of sorgoleone in *Sorghum bicolor* cv. SX17

Treatment[a]	Root		Sorgoleone	
	(mg dw ± sd)[b]	% control	(μg/mg root dw ± sd)[b]	% control
Control	55.5 ± 4.0 ab	–	16.4 ± 0.2 a	–
Chitin	51.5 ± 2.1 ab	92.8	16.5 ± 1.3 a	100.6
Velvetleaf	44.8 ± 8.1 b	80.7	18.8 ± 0.6 a	114.6
Actigard	50.7 ± 2.7 ab	91.3	11.5 ± 1.2 b	70.1
Messenger	71.3 ± 16.3 a	128.5	14.4 ± 3.6 ab	87.8
ProAct	50.7 ± 0.5 ab	91.3	18.3 ± 0.6 a	111.6

[a]Concentrations of chitin, velvetleaf extract, Actigard, Messenger, and ProAct were 10, 0.5, 0.5, 0.75, and 0.75 mg/ml , respectively
[b]Numbers in columns followed by the same letter are not different at $P < 0.05$ according to Tukey's test

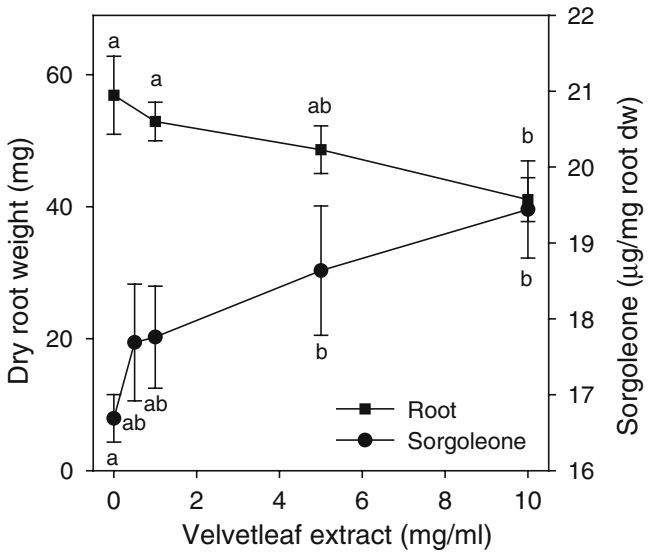

Fig. 6 Effect of velvetleaf root extract on root formation and the production of sorgoleone in *Sorghum bicolor* cv. SX17. Sorgoleone was extracted after 7 days of growth in the dark at 30°C. Means with the same letter above (root) or below (sorgoleone) the symbols are not different at $P < 0.05$ according to Tukey's test

Discussion

As far as it is known, all sorghum cultivars produce sorgoleone, though they have been reported to vary considerably in the total amount of sorgoleone, ranging from 0.67 to 17.8 mg/g root FW in one study (Nimbal et al. 1996a). However, other studies have shown that there is very little variation in sorgoleone amount across germplasms (Hess et al. 1992; Czarnota et al. 2003a). Furthermore, Hess et al. (1992) also demonstrated that the production of sorgoleone was not dependent on their respective susceptibility or resistance to *Striga*.

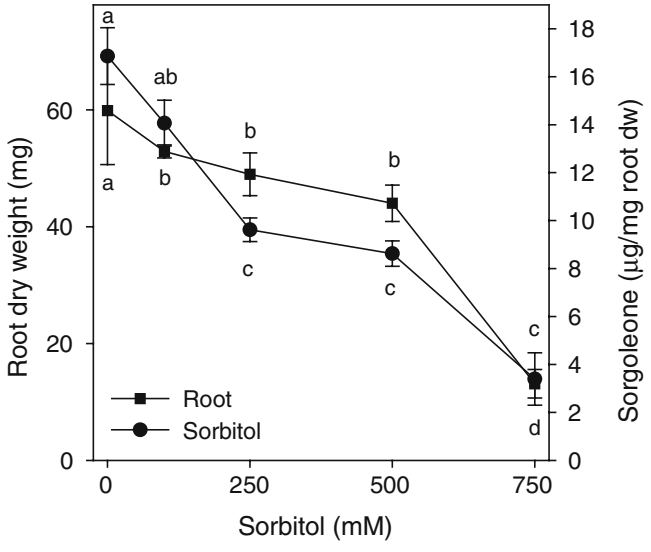

Fig. 7 Effect of osmotic stress on root formation and the production of sorgoleone in *Sorghum bicolor* cv. SX17. Osmotic stress was induced by lowering the water potential of the solution with sorbitol (see Materials and methods). Sorgoleone was extracted after 7 days of growth in the dark at 30°C. Means with the same letter above (root) or below (sorgoleone) the symbols are not different at $P < 0.05$ according to Tukey's test

It has been well documented that sorgoleone is exuded as an oily droplet at the tip of the root hairs (Fig. 1a). At the ultrastructural level, sorghum root hairs are highly, physiologically active with a complex network of smooth endoplasmic reticulum and possibly Golgi bodies. Small globules of cytoplasmic exudate were also observed to deposit the oily material between the cell wall and the plasma membrane near the tip of root hairs (Czarnota et al. 2003b). In this study, sorgoleone started exuding from the tip of root hairs after these nascent secretory cells have stopped elongating. This is probably due to resource allocation within the root hair, where most of the energy is devoted to development until it reaches its maximum length, after which sorgoleone biosynthesis is initiated.

Hess et al. (1992) indicated that sorgoleone production is quite sensitive to environmental conditions, particularly to moisture. The negative impact of high moisture was also reported by Yang et al. (2004) and is corroborated by this study. This is attributed to the inhibition of root hair formation under high moisture conditions (Yang et al. 2004). Root hair development can be restored by ethylene under such conditions. However, ethylene appears to be only one of the components regulating root hair initiation and elongation in sorghum, whereas it is a key factor in Arabidopsis (Dolan 2001). Sorghum root development was also reduced under hypoxic conditions, but the lack of oxygen did not affect root hair formation to a great extent (Yang et al. 2004).

Optimum root growth and sorgoleone production was at 30°C and decreased rapidly at temperatures below 25°C and above 35°C. Thus, the allelopathic potential of this cover crop may be compromised in the field under certain temperature ranges.

In contrast to the positive effect of increasing mechanical pressure on root biomass, none of the light treatments affected root formation. However, sorgoleone levels were greatly repressed by blue light and slightly reduced by red light, relative to dark grown control seedlings. The physiological basis of the repression of sorgoleone production remains to be determined, but could be the result of phytochrome regulation of the biosynthetic pathway or a change in carbon allocation.

In preliminary experiments, root formation was enhanced when the top lid of the Petri dish was placed upside down (i.e., when the surface of the top lid applied some pressure over the seedlings). Applying mechanical pressure over the top of developing seedlings resulted in more than a doubling of the root biomass produced by developing seedlings (Fig. 4). The greatest increase was observed in adding 1 g cm^{-2} of pressure over the seedlings, and the positive modulation being progressively lower with higher weights. However, the root biomass

accumulation did not translate in a greater amount of sorgoleone produced per mg of root dry weight (Fig. 4), suggesting that the treatment did not stimulate sorgoleone production per unit of root. It should be noted that applying mechanical pressure over germinating seedlings may enhance root biomass by mimicking the effect soil compaction and weight have on developing roots in natural settings.

Sorgoleone production was slightly stimulated at lower pH, suggesting that the allelopathic potential of sorghum may be more effective in acidic soils than in those with more alkaline pHs. In this laboratory experiment, the overall levels were reduced relative to the amount extracted from seedlings grown in water without buffer (Fig. 5). The cause for repression of sorgoleone production associated with the presence of phosphate-citrate or potassium phosphate buffers is unclear, but it may be the results of stress caused by the buffers since sorgoleone is affected by increasing osmotic stress (Fig. 7).

Sorgoleone has some antifungal activity (Suzuki et al. 1998) and its production may respond to pathogenic infections. Attempts to stimulate sorgoleone biosynthesis by eliciting plant defense mechanisms provided mixed results. Exposing plants to Actigard, a salicylic acid-like elicitor of SAR (Inbar et al. 1998), significantly repressed sorgoleone production (Table 2). Treating plants with chitin, which is known to induce the expression of SAR genes (Hahn 1996; Zhang et al. 2002), or Messenger, which is known to induce expression of plant defense genes such as PR1, PR2, PDF1.2, and Thi2.1 (Biesgen and Weiler 1999; Brown et al. 2003; Epple et al. 1998), did not have a significant effect on sorgoleone production. Plants exposed to the more complex harpin-based elicitor ProAct had only slightly higher levels of sorgoleone. The difference in the effect of the two harpin-based elicitors (Messenger and ProAct) on sorgoleone production remains to be explained.

The production of phytotoxins is known to respond when certain allelopathic plants grow in the presence of competing plants. For example, the production of two rice allelochemicals (a flavone and a cyclohexenone) is stimulated in the presence of barnyardgrass (Kong et al. 2004). In the case of sorghum, Einhellig (1986) has shown that a water-soluble extract of velvetleaf affects sorghum growth. In response to this stimulus, sorghum seedlings produced increasing amounts of sorgoleone in a dose-dependent manner (Fig. 6). This response is all the more noticeable in that it is not accompanied with an increase biomass, suggesting that the production of sorgoleone may be elicited by the velvetleaf extract. The increase is not attributed to changes in the water potential of the solution, since the experiment with sorbitol demonstrated that both root growth and sorgoleone biosynthesis were repressed with increasing osmotic stress.

In conclusion, sorgoleone production is constitutive to the physiology of mature root hairs of sorghum. No differences in sorgoleone levels were observed during the early stages of seedling development and the biosynthesis of this allelochemical either did not change or was negatively affected by most of the stimuli used in this study. However, temperature appears to be one of the most important environmental factors affecting sorgoleone production, suggesting that the overall allelopathic potential of sorgoleone may be affected in the field when temperatures are lower than 25°C or exceed 35°C. Finally, sorghum seedlings produced more sorgoleone when grown in the presence of a water-soluble velvetleaf root extract. While the concentrations required to stimulate sorgoleone synthesis are relatively high, the more complex plant–plant interactions that occur when the rhizospheres of these two species interact in a natural environment may result in a similar elicitation of sorgoleone production.

Acknowledgements I am thankful for the excellent technical assistance provided by J. Howell and S. B. Watson.

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